through regulation of POMC, a process much more pronounced in keratinocytes than in other cell types, p53 ensures our well being in many different and complementary ways.

As UV is probably the most prominent naturally occurring environmental carcinogen, it is quite conceivable that this newly described role of p53 has been an important driving force in the selective pressure to maintain p53 function during evolution. In this regard, it is noteworthy that a common p53 gene polymorphism, affecting amino acid position 72 of p53, exhibits a striking geographical bias, with the allele encoding proline at position 72 becoming much more prevalent as one approaches the equator (Beckman et al., 1994). It is tempting to speculate that the 72Pro isoform of p53 is a more competent inducer of POMC transcription, thus driving strong evolutionary selection in its favor in heavily sun-exposed areas.

Another intriguing aspect of the discovery of the p53-POMC pathway relates to the current efforts to stimulate p53 function by small molecules (Vassilev, 2007). One may imagine designing skin lotions that moderately activate p53 in our keratinocytes—enough to trigger the suntan response safely—without causing DNA damage and hence avoiding the health risks otherwise inherent in UV exposure. It is conceivable that the p53-regulated pigmentation mechanism will become particularly handy in the coming decades of escalating environmental pollution and global warming.

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N-WASP Generates a Buzz at Membranes on the Move

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The fast-growing ends of actin filaments push against membranes to create cell-surface protrusions and to propel the movement of membrane vesicles. Co et al. (2007) now show that the neural Wiskott-Aldrich syndrome protein (N-WASP) mediates dynamic attachment between membranes and the growing ends of actin filaments to sustain membrane movement.

Actin polymerization drives protrusions at the cell surface and the propulsion of membrane vesicles (Pollard and Borisy, 2003). In these processes, actin subunits are added to the barbed end of a filament—the same end that pushes against the membrane. Yet, if the barbed end abuts the membrane, how are actin subunits added to it? It has been proposed that thermal motion of the filament might create a gap between the membrane and the barbed end that allows the binding of an actin monomer (Mogilner and Oster, 1996). Restoring the position of the filament would then provide the power to push the membrane. This model also pre-

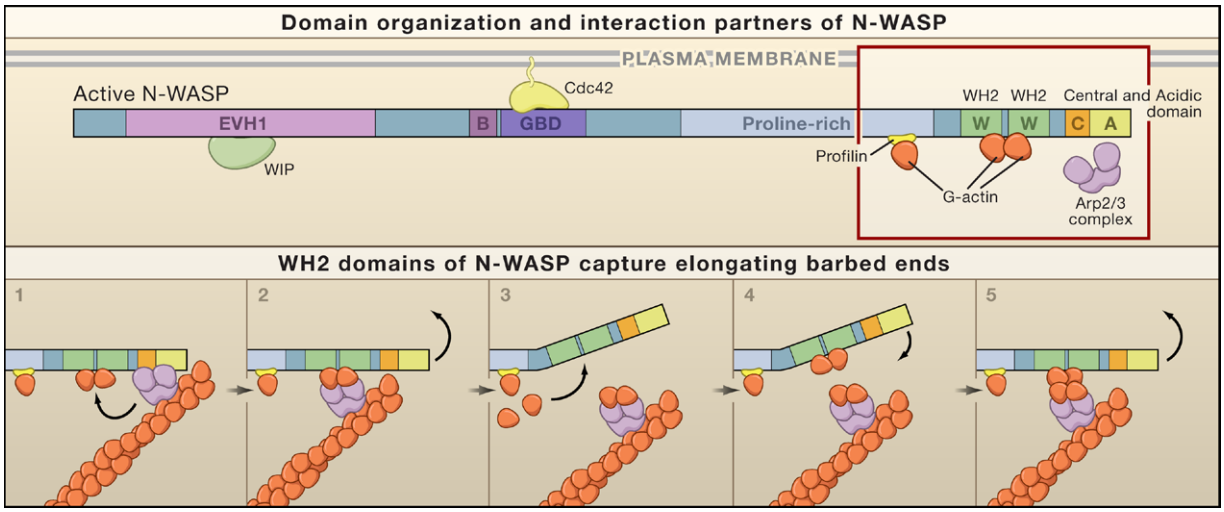

Figure 1. N-WASP and Actin Filament Elongation

(A) Domain organization and some interacting partners of N-WASP. Domains: Ena/VASP Homology 1 (EVH1) domain strongly binds to WIP (WASP-interacting protein) family proteins, which are thought to stabilize the conformation of inactive N-WASP. The basic domain (B) binds to PIP_2 and contributes to N-WASP activation and recruitment to the membrane. The GTPase-binding domain (GBD) binds to GTP-bound Cdc42, which leads to N-WASP activation and recruitment to the membrane through the Cdc42-bound prenyl tail. The proline-rich domain can bind to many proteins including profilin, which likely recruits actin monomers for the actin nucleation and polymerization reactions. The two WASP homology 2 (WH2) domains bind to actin monomers and, as shown by Co et al. (2007), to the barbed ends of actin filaments. Central (C) and acidic (A) domains bind and activate the Arp2/3 complex by inducing a major conformational change.

(B) The insertion of actin monomers at elongating barbed ends. Panels show individual events during nucleation and elongation of actin filaments: (1) The central and acidic (CA) domain module of N-WASP binds and activates the Arp2/3 complex, and the WH2 domains bind to actin monomers; (2) The Arp2/3 complex binds to WH2-associated monomers and dissociates from the CA module; the barbed end of the newly formed nucleus is attached to the WH2 domains of N-WASP; (3) due to the low affinity predicted for the WH2-barbed end interaction, WH2 domains frequently dissociate from the barbed end; (4) new monomers bind to the WH2 domains, some of which might come from the pool associated with the proline-rich domain; (5) WH2 domains rebind the barbed end and simultaneously add new actin subunits. The filament grows though repetitions of this cycle (steps 3–5).

dicts that there is an optimal angle between the membrane surface and a filament at which the filament is both easily deflected yet retains the power to push. However, the spatial organization of actin filaments—and therefore the angle between the membrane surface and growing filaments—can vary. Actin filaments can form either parallel bundles (as are found in filopodia) or branching networks (as are found in lamellipodia or in the "comet tails" that drive the motility of vesicles and some intracellular parasites). In the case of filopodia, actin filaments abut the membrane orthogonally and do not have much room for vibration. Also, tilted filaments in branched networks might eventually miss their target during elongation, especially if the target is a small vesicle.

Therefore, an alternative model has been proposed postulating that special end-tracking motors assist in the insertion of actin monomers between the barbed end of the filament and the membrane, while keeping the filament nearby (Dickinson et al., 2004). This gives the filament the freedom to grow, but not to escape. The formin family proteins (Zigmond, 2004) that bind barbed ends, while allowing for their elongation, are potential examples of end-tracking motors. However, formins usually function in association with actin filament bundles. It has been less clear which proteins might restrain growing filaments in branched actin arrays. Co et al. (2007) now report that the neural Wiskott-Aldrich syndrome protein (N-WASP) directs the addition of actin monomers and promotes attachment between the membrane and growing actin filaments in branched actin arrays (Figure 1).

N-WASP activates the Arp2/3 complex, which then nucleates actin (Pollard and Borisy, 2003). The C-terminal domain of N-WASP (containing two WASP homology 2 [WH2] subdomains and the central [C] and acidic [A] subdomains) is necessary and sufficient for Arp2/3 activation. The central and acidic (CA) module binds to the Arp2/3 complex and induces a conformational change leading to Arp2/3 activation. Meanwhile, the WH2 subdomains of N-WASP bind to actin monomers that are then added onto Arp2 and Arp3 of the activated Arp2/3 complex, which has a conformation similar to the barbed ends of actin filaments. This completes the formation of the actin nucleus. It has been thought that the role of N-WASP ends here and that N-WASP dissociates from Arp2/3, as well as from the newly added monomer, to allow for filament elongation.

In contrast to this assumption, Co et al. show that N-WASP holds onto actin filaments even after the nucleation event, and that this occurs via dynamic interaction between the WH2 domain of N-WASP and the barbed end of the actin filament. Using vesicle motility in cytoplasmic extracts as a model system, this group found that a single WH2 domain was necessary and sufficient to target N-WASP

to the vesicle surface where the actin tail was attached. Furthermore, Co et al. reconstituted actin-based "comet-tail" motility in vitro with purified components using lipid-coated beads bound to activated Cdc42, thereby mimicking the recruitment and activation pathway of N-WASP that occurs in vivo. Strikingly, the introduction of point mutations in the WH2 domain that interfere with the binding of actin monomers, but not with Arp2/3 activation, caused the actin network to detach from the beads over time. Additional experiments demonstrated that WH2 domains do not bind directly to a pre-existing barbed end but require the presence of actin monomers.

In order to keep vesicles moving, the link made by N-WASP between the membrane and actin filaments in the tail should be dynamic. This might be due to the low affinity of binding that is predicted to occur between WH2 domains and actin. This association-dissociation mechanism would appear to be a less robust strategy for retaining barbed ends than the processive association of formins with elongating barbed ends (Zigmond, 2004). Yet, high concentrations of N-WASP at the membrane likely solve the problem. On the other hand, nonprocessive capture of barbed ends by N-WASP may be advantageous in the context of dendritic arrays, in which individual filaments frequently leave a particular area because of capping or lateral drift, and as a consequence N-WASP needs the capacity to frequently swap filaments.

Interestingly, the ability of N-WASP to serve as a dynamic linker has been predicted much earlier, between the actin comet tail and the surface of bacterium Shigella, which displays rocketing motility inside infected cells (Egile et al., 1999). These authors also predicted that the vasodilator-stimulated phosphoprotein (VASP) plays a similar role for rocketing motility of another bacterium, Listeria. Co et al. have provided experimental evidence that N-WASP functions as an end-tracking motor, but VASP may also re-enter the scene as having this capacity. The biochemical activities of VASP family proteins include actin filament crosslinking and protection of barbed ends from capping (Krause et al., 2003; Barzik et al., 2005). In cells, VASP family proteins localize to sites rich in elongating barbed ends: the leading edge of lamellipodia, tips of filopodia, and ends of stress fibers, where they promote filament elongation (Bear et al., 2002). It was recently observed that both N-WASP and VASP family proteins have similar tripartite C-terminal domains (Chereau and Dominguez, 2006). The VASP C-terminal domain contains a G-actin-binding domain (GAB), an F-actin-binding domain (FAB), and a coiled-coil domain. Despite different names, GAB and FAB are very similar to the WH2 and C domains of N-WASP, respectively. Therefore, VASP may promote elongation of barbed ends if FAB binds an actin filament close to the barbed end, similar to how the CA region binds the Arp2/3 complex, and GAB puts a monomer on the barbed end, similar to how WH2 adds a monomer to the Arp2/3 complex. The coiled-coil subdomain mediates VASP oligomerization and allows for actin filament crosslinking, which in theory might help VASP to gain processivity. As shown for N-WASP by Co et al., it will be interesting to test whether VASP links actin filaments to the membrane.

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